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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : B01D 25/12, 63/00, B01J 19/00 B01L 9/00, 11/00 C08F 283/00, 285/00 C07H 21/00, C07K 1/06, 5/00 C07K 7/00, 15/00, 17/00 C08L 89/00, C12Q 1/00 C12P 19/04, 19/38, 21/06 G01N 21/00, 33/00, 33/543 G01N 33/544, 33/545, 33/546 G01N 33/552	**(11) International Publication Number:** **WO 94/05394** **A1** **(43) International Publication Date:** 17 March 1994 (17.03.94)

(21) International Application Number: PCT/US93/08267

(22) International Filing Date: 2 September 1993 (02.09.93)

(30) Priority data:

| 07/939,065 | 2 September 1992 (02.09.92) | US |
| 08/079,741 | 18 June 1993 (18.06.93) | US |

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(81) Designated States: AU, CA, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: SPECIFICATION METHOD AND APPARATUS FOR PEPTIDE SYNTHESIS AND SCREENING

(57) Abstract

manufacture and assay polymer film plates at nodes or links immobilized porous points of anchor are terminants received for use in the plate 5. The plate 5 is employed with a vacuum block system (46, 47, 48) to assist in washing, deprotection of protected FMOC protected amino acids, and screening of immobilized, synthesized hexapeptides, for example, to de...

SPECIFICATION
METHOD AND APPARATUS FOR PEPTIDE
SYNTHESIS AND SCREENING

BACKGROUND OF THE INVENTION:

This invention relates to methods and apparatus for preparing a non-volatile, reusable, Addressable Synthetic Biopolymer Combinatorial Library (ASBCL) having known sequences at identifiable designated addresses arrayed on a permanent substrate, which library is rapidly creatable by a unique and simple slotted block system. The invention also relates to the use of ASBCLs to screen for sequences having biologic, biochemical, biomedical or therapeutic activity relative to a specified target. The invention permits rapid optimization of leads for identification of active components when applied to the specific area of peptides. The invention may be termed PILOT, for Peptide Identification and Lead Optimization Technique.

Moderate length peptides have attracted considerable research and commercial interest by virtue of the properties some exhibit in enhancing, blocking or otherwise affecting the activity of receptors, microbes, and other molecules deemed biologically significant. Specifically, hexapeptides have proven to have a sufficient chain length to block much larger molecules such as receptors, enzymes and antibodies. Thus, synthetic and natural hexapeptides have exhibited diverse therapeutic properties, among them: Antimicrobials with minimum inhibitory concentrations an order of magnitude less than known natural antimicrobial peptides; bactericides; antivirals; activity as antigenic determinants; and the like. The problem is that there are

Indeed if the selection were made from all of the combinations the number amounts to 4.096 billion. Since

1   significant targets, preparing a complete suite of just
2   64m L-hexapeptides and assaying activity for each of the
3   millions of targets is, practically speaking, an infinite,
4   and therefore, impossible, task.
5   Accordingly, the Synthetic Peptide Combinational
6   Library (SPCL) approach has recently resulted in a
7   manageable approach to the problem of screening for a
8   unique hexapeptide among the 64m that is the most active
9   for a given target. In order to be feasible, libraries of
10 large numbers of hexapeptides, on the order of 100,000 or
11 so at a time, must be prepared in quantities sufficient to
12 result in a positively determinable reaction.
13   There are currently five basic library techniques
14 offered: viral approachs (originated by George Smith of
15 LSU, and by Cetus and Affymax independently); the Chiron
16 Geysen polyethylene pin system; the Houghten approach
17 using Tea-bags; the Selectide bead approach; and the
18 Affymax Chip approach. The latter four have distinct
19 advantages over the viral approach in which peptide
20 libraries are displayed by bacteriophages (viruses that
21 prey on bacteria). A short degenerate oligonucleotide
22 encoding all combinations of a short peptide sequence is
23 cloned into Gene III or VIII of a filamentous phage and
24 expressed on the phage surface. Recombinant phage are
25 screened with the target molecule (e.g. receptor), and
26 phage expressing a certain peptide that binds to the
27 target are identified. Nucleotide sequence analysis of
28 the recombinant Gene III or Gene XIII identifies the
29 peptide sequence displayed by the binding phage.
30   The problem with the viral approach is that the range
31 of peptides is limited to those tolerable by virus and E.
32 Coli. That is, only a limited suite of peptides can be
33 produced from among the 64m possible hexapeptides, and
34 likewise for the even greater numbers of longer peptides.
35 Additionally, only L- amino acids are allowed, and each
36 individual hexapeptide of the library is produced within
37 the phage as fusion products. This reduces the
38 flexibility of the sequences, and may mask them entirely.
39   Methods for synthesis and display of peptides on

1   surfaces as well as techniques for binding from partial
2   sequences were reviewed by H. Mario Geysen in Geysen, H.M.
3   et al, Synthetic Peptides as Antigens, Wiley Chichester
4   (Ciba Foundation 119), 130-149 (1986), shown in U.S.
5   Patent 4,833,092 (19____).   Geysen used functionalized
6   polyethylene pins clustered to fit 96 hole microtiter
7   plates.   This Chiron system also relies on the method
8   shown in Rutter-Santi patent 5,010,175 of preparing
9   peptide sequences by providing constituent amino acids in
10 concentrations relative to each other based on their
11 relative coupling constants so that the resulting peptide
12 mixture contains peptides in equimolar amounts.   Chiron
13 reports that its recent patent 5,194,392 entails
14 synthesizing up to 1000 peptides a day on special pins,
15 evidently a reference to the Geysen pin system of
16 4,833,092.   The peptides can be used to "map" regions
17 called epitopes in any protein of interest, such as
18 antigen regions that trigger an immune response by T-
19 cells.
20   The Selectide bead approach uses vast quantities of
21 spherical crosslinked polymer beads (Millipore or
22 Cambridge Research Laboratories polyacrylamide beads or
23 Rapp Tentagel polystyrene) divided into 20 equal piles,
24 each of which then has a different L-amino acid coupled to
25 all the beads in the pile.   The bead piles are then
26 combined and thoroughly mixed. The resulting single pile
27 is again divided into 20 different piles, each of which is
28 reacted with a different one of the 20 different L-amino
29 acids. This Divide, Couple and Recombine process (DCR) is
30 repeated through six reactions to produce hexapeptides
31 bound to the beads.   The beads are then screened against
32 a "target" molecule which is marked with a conjugated
33 enzyme, such as horseradish peroxidase.   The target
    " target" to active hexapeptide(s). The bead is rendered

37 the beads, and then the visually identified beads are
38 picked out with tweezers. The peptides on the beads are

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1   and soluble versions produced in a synthesizer. The
2   initial screening (locating the target bead(s)) takes only
3   days, the makeup of each identified hexapeptide is
4   unknown, and the analysis and synthesis for confirmation
5   and further work takes much longer.
6   The Houghten (Iterex) Tea-Bag method, shown in U.S.
7   Patent 4,631,211, employs methylbenzhydrylamine (MBHA)
8   polystyrene beads in a number of foraminous containers,
9   e.g. porous polypropylene bags (Tea-Bags), to prepare a
10 truncated SPCL. In order to shorten the processing time,
11 the Tea-Bag process employs partially known, partially
12 undetermined hexapeptide sequences in repeated screenings,
13 followed by iterative resynthesis to replace the unknown
14 AA sequence positions with known AAs, i.e., $A-O_1O_2O_3XXX$, $A-$
15 $O_1O_2O_3O_4XX$, etc. The method works on the assumption that a
16 biologically significant response can be detected from a
17 solution which contains hundreds of thousands of inactive
18 components.
19   The Tea-Bag process typically uses 18 of the 20 L-Aas
20 (cysteine and tryptophane are omitted in the initial
21 library for ease of synthesis), starting with 104,976
22 combinations of non-determined tetrapeptide resins (XXXX-
23 peptide resins) in 324 aliquots, and adds the 324 known
24 dipeptide sequences (18^2) in the terminal two positions.
25 For epitope determination of antibody binding, the 324
26 pools are screened to see which best inhibits binding of
27 the target antibody with its natural antigen. The most
28 active amino terminal dipeptide sequences are then
29 incorporated into a further set of 20 pools in which the
30 third residue is varied. These are rescreened for low IC.
31 The most active sequences are again reincorporated
32 iteratively to define positions 4-6 to finally obtain a
33 characterized active hexapeptide.
34   The foraminous container of the Tea-Bag must retain
35 the solid phase beads, yet have a sufficient number of
36 openings to permit ready entrance and exit of solvent and
37 solute molecules at the reaction temperature, but bar exit
38 of the solid phase. While the synthesis is the standard
39 Merrifield technique, new linking groups that attach the

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1   X_n-peptide to the styrene bead supports are disclosed.
2   This process can be characterized as not calling for a
3   continuous support, and it is not addressable.

4   The Affymax "chip" approach described in PCT
5   publication WO90/10570, and in Fodor, P.A. et al, Science,
6   251 (1991) 767, is a method for multiple peptide synthesis
7   on a solid support which uses synthesis and flourescent
8   detection on the silica surfaces of flow through cells,
9   photolabile protecting groups and photolithographic
10   masking strategies to make arrays. Photolabilely-blocked
11   amino groups are chemically attached (bonded) to a silicon
12   chip, then irradiated through a patterned mask to
13   selectively remove the blocking groups in a pre-arranged
14   pattern. An amino acid will bond by addition only to the
15   irradiation exposed areas. Additional masks are imposed
16   and radiation applied as a prelude to adding second amino
17   acids. Each amino acid added can include a blocking group
18   so that further addition to that site occurs only after
19   irradiation unblocking. Repeating the process with plural
20   masks builds location specific polypeptides. When the
21   chip is exposed to the target molecule, it may stick to
22   one or more locations. By checking coordinates on a map
23   of the chip, the peptide is identified. However, this
24   process does not work with target molecules stuck to, or
25   part of, cells, and there are exposure problems during
26   processing, i.e., some AA's are light sensitive and cannot
27   be used. Further, the reactions at the surface are not
28   complete; for example, where reaction completion is only
29   90%, by the 6th iteration to obtain a hexapeptide, only
30   half of them will be made properly.

31   There is clearly a need in the art for a peptide
32   synthesis and screening process that is rapid and
33   accurately identifies the active peptides from amongst
34   those in an extended, reusable SPCL. Accordingly, it is

Addressable Synthetic Biopolymer Combinatorial Library
(ASBCL) having known amino acid sequences at identifiable
designated addresses arrayed on a permanent substrate so

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1   rapid screening of target receptors and molecules by use
2   with the Peptide Identification and Lead Optimization
3   Technique (PILOT) which employs a simple slotted block
4   system for rapid multiple amino acid addition reactions to
5   build peptides of known sequences at identifiable
6   designated addresses in an X-Y coordinate array on a
7   variety of planar substrates, and preferably uses sintered
8   polyolefin discs having thereon ultra-thin HPMP films,
9   called thin-film HPMP Winks, in plate, slotted block and
10   vacuum block assemblies, to permit display of assembled
11   synthon molecules in an unhindered, near-aqueous
12   environment, and thereby permit high quality peptide
13   ligand synthesis, high ligand loading, efficient binding
14   of radioactive target molecules and facile removal of
15   unbound targets by suction washing, and repeated
16   regeneration thereof.
17

18   **SUMMARY OF THE INVENTION:**
19   The invention comprises methods and apparatus for
20   preparing non-volatile, reusable, Addressable SBCLs
21   (ASBCLs) or SPCLs (ASPCLs), having known arrayed dipeptide
22   amino acid sequences incorporated at any desired and known
23   position within any biopolymer (e.g., polypeptide)
24   sequence of length producible by chemical synthesis
25   methodology, in which up to 6 positions (typically 4) may
26   be composed of mixtures of residues, the remaining
27   positions comprising specified amino acids. The arrayed
28   peptides are identifiable from designated addresses
29   provided on a permanent, reusable substrate-containing
30   plate which permits creation of an ASPCL, typically within
31   a day. While the discussion herein is with reference to
32   hexapeptides by way of example, the principles of the
33   invention are applicable to any binding determinant
34   biopolymer to produce an ASBCL for interaction with any
35   biologically significant target. These library peptides
36   are also termed ligands.
37   The ASBCLs of this invention can uniquely identify
38   the binding determinant biopolymer, e.g. an active
39   hexapeptide, at a unique X-Y coordinate axis upon bonding

1   with or adhering to a fluorescently labeled, radio-labeled
2   or enzyme-linked target molecule or receptor, e.g., in
3   solution flowed into contact with the ASPCL-bearing
4   substrate. The amino acids and peptide sequences are
5   substantially equimolar concentrations on the substrate,
6   so good quantitative activity is determinable by the
7   fluorescent or radio intensity, or by the optical density
8   of the dye product formed in the enzyme linked probing.

9   In addition, the hexapeptide array is permanently
10 bound to the substrate i.e., is non-volatile, and at each
11 substrate site on the array plate on the order of 1μ mole
12 or more can be bonded. The target molecule can be washed
13 off the substrate plate so that it can be reused
14 repeatedly, particularly for diagnostic testing, as well
15 as rapid active peptide screening on a wide variety of
16 target receptors. For example, a preselected library of
17 peptides, or any other condensation chemistry-based
18 screening agent, may be permanently bonded to a substrate
19 as a diagnostic tool. One example involves exposing an
20 ASPCL plate of this invention to one or more aliquots of
21 a serum which requires diagnosis, and then visualizing
22 binding by passing a flourescently or radiolabeled anti-
23 IGG antibody over the ASPCL plate. Consequently, one or
24 more conditions, such as the presence of antibodies to
25 HIV-1, or the presence of other viral infections can be
26 rapidly and simply diagnosed.

27 More broadly considered, the peptide may be any
28 biopolymer. Thus the term ASBCL applies to the library
29 on the identifiable designated addresses arrayed on the
30 permanent, reusable substrate plate. It should be
31 understood that the term "substrate" as used herein
32 includes broadly but is not limited to: a) polyolefin
33 plate alone, or, preferably, an activated plate carrying
34 a plurality of substrate discs; b) a plate with a head or

functionalized or bare, spacer receptor substrates; and
d) reacted substrates i.e. such substrates above with one
or more Aas or peptides linked target

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1   　　　The apparatus system of this invention comprises
2   employing an inert substrate support plate, such as a
3   polyolefin polymer, having a plurality of discrete sites
4   (such as holes for retainingly engaging removable
5   substrate discs, called winks, or small, well-like,
6   shallow flat-bottomed circular or square depressions) in
7   a spaced array, e.g., 4x4, 10x10, 16x16, 20x20, 40x40,
8   100X100, 400x400, or any other desired number. The wink-
9   holding plate array is presently preferred. Each of the
10 sites are permanent uniquely addressable locations for
11 assembly of the biopolyer chains or attachment of
12 preactivated molecules. Each site includes an amino
13 functionalized substrate media such as a sintered
14 polyolefin (polyethylene or polypropylene) disc, glue-
15 bonded beads, or chemically grafted polymeric films, which
16 may be gel-type films. Any conventional peptide
17 substrate, media, or addition chemistry-based agent
18 substrate, may be used.
19 　　　The preferred substrate is a 1/4" diameter sintered
20 polyethylene disc of approximately 1/8" thickness, which
21 is coated with an ultra-thin Hydrophilic Polar Multi-
22 functionalized Polymer (HPMP) film, herein called a
23 "wink". The film and methods of anchoring to the
24 polyolefin are disclosed in more detail in our co-pending
25 priority application U.S. Serial No. 08/019,725, the
26 disclosure of which is incorporated by reference herein to
27 the extent needed.
28 　　　The film is preferably carboxymethyl dextran carbodi-
29 imide coupled to the polyolefin disc surfaces after
30 functionalizing them with a diamino-substituted
31 polyethylene glycol spacer arm.
32 　　　Another example of a film is a hydrolyzed or partly
33 hydrolyzed chitin (herein chitin/chitosan) having from
34 about 1 to about 100 sugar moieties per amino group which
35 may be coupled to the polyolefin disc surface with mono-
36 amino substituted polyolefin glycol spacer arms after
37 functionalizing most of the amino groups with protective
38 functionalities such as t-Boc or Fmoc that can be removed
39 later. The degree of alkaline hydrolysis of the chitin

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1   controls the solubility of the resulting chitosan. The
2   amino group and hydroxyl on the sugar moieties permits the
3   chitin/chitosan to be bifunctional providing a wide
4   variety of tether linkages to ligands through either or
5   both types of groups. The amino groups provide a
6   controllable positive charge that may be advantageous in
7   certain affinity binding environments. The polymer film
8   molecular weight may range from about 50,000 to about 1
9   million.

10   The films permit display of assembled synthon molecules
11   (ligands) in an unhindered, near aqueous environment.
12   These substrates permit high quality peptide (ligand)
13   synthesis, high ligand loading in the film in the range of
14   50-100 nmole loading, efficient affinity binding of
15   radioactive or flourescent labeled target molecules, and
16   easy removal of unbound targets by suction washing, and
17   repeated regeneration and reuse of the library. The
18   substrate discs are easily pressed into and removed from
19   holes bored partway through a substrate carrier plate,
20   which preferably includes at least one smaller through-
21   hole to permit rapid and thorough suction removal and
22   washing of the array of substrate discs in the carrier
23   plates. A vacuum base plate is used with the array carrier
24   plate to facilitate the excess target solution removal and
25   washing steps in the common synthesis and probing steps.

26   Examples   of   bead   type   substrates   are
27   polydimethylacrylamide (PDMA) particles, silica beads,
28   MBHA polystyrene beads, and the like, which are glued to
29   the substrate areas of the support plate. The presently
30   preferred bead substrate is Kieselguhr-encapsulated PDMA
31   particles (Pepsyn-K from Millipore Corp.), secured to a
32   polyethylene plate with a low temperature (<100 degrees C)
33   hot-melt polyethylene adhesive. The preferred polymeric
     film is chemically grafted to the surface of the wells by

     Two methods of attachment of amino-functionalized
     polymers to form substrate areas on the plates are

1 invention; in situ polymerization (disclosed in detailed
2 examples); and bonding of a pre-polymerized material to
3 activated areas on the plate (disclosed in general). The
4 first polymerizes acryloylated monomers and crosslinking
5 agents onto acryloyl groups attached to the areas of the
6 polyolefin surface (plate activation). This establishes
7 a gel-type polymer covalently grafted into the
8 depressions. This polymer, since it may possess low
9 structural strength, i.e. it need not have high structural
10 strength, can be prepared from monomers at low
11 concentration and with a low molar percentage of cross-
12 linking. The resulting gel substrate materials are
13 therefore highly permeable to proteins, thus greatly
14 improving the sensitivity of detection. The gel film may
15 then be amino functionalized.
16   A spacer arm derivative is attached to the functional
17 amino groups of the resultant films. These spacer arms,
18 which are also used for the glued beads, increase
19 sensitivity since they reduce unfavorable steric and
20 electronic interactions between the incoming tagged
21 protein and the polymer backbone. An ideal spacer is non-
22 hydrophobic, incapable of forming aggregates by hydrogen
23 bonding, and typically longer than 10Å. A variety of
24 materials, including functionalized polyethylene glycols,
25 sugars, and short natural and unnatural peptides may be
26 used as spacers.
27   The second (pre-polymerized) method of attachment of
28 functional polymers involves attachment of preformed
29 amino-functionalized polymers to the designated areas on
30 the inert (polyolefin) support plate itself, or on the
31 sintered polyolefin winks (discs) which are retained in
32 the plate. The preformed polymer itself serves as a
33 spacer arm, and access of proteins is improved as compared
34 to the in situ gel type polymers. Examples of preformed
35 amino-functionalized polymers include polyethyleneimine,
36 polyallylamine, long chain functionalized sugars (e.g.
37 dextrans and chitosans), polyamino acids (e.g. poly-L-
38 lysine) and the like. They can be coupled to acid
39 chloride activated plate areas by reactions of the type

1　described herein. We prefer to use a 500,000 MW dextran
2　that is amino-functionalized pre- or post-attachment to
3　the plate area, or to the winks.

4　　　　For the array preparation steps, an elastomeric
5　sealing gasket having a plurality of holes or slots
6　therethrough aligned with the substrate areas is placed
7　over the substrate array plate or wink carrier plate, and
8　a slotted block is placed over the gasketed plate so that
9　individual slots align with rows of substrate areas. When
10　the wink carrier plate is used, a solid non-apertured
11　gasket is placed below the wink plate to seal the through-
12　holes, and then placed on a base plate. The assembly of
13　the plate, slotted block and appropriate gasket(s) is
14　clamped together for use.

15　　　　Since each area is part of a predetermined array,
16　each defined substrate area has a unique X-Y coordinate
17　address, such as: Row 1, position 1; Row 2, position 20;
18　Row 3, position 78; to Row X_n, position Y_m. Any desired
19　address system may be used, such as sequential numbers for
20　each succeeding area, dual alpha system (AA, AB, AC etc.),
21　or alpha numeric (A1, A2...B1, B2...).

22　　　　The slotted block has a height sufficient to provide
23　a well of sufficient volume to receive reaction solution
24　having selected moieties for bonding with the exposed
25　substrate address area, or for reaction with a previous
26　moiety. Each well can receive a different reactant, e.g.
27　a blocked AA, so that each row has a different A_n position
28　AA. Next, the reactants are removed from the slots in the
29　block, e.g. by decanting or suction, then the amino
30　blocking group removed by a deblocking agent, e.g.
31　piperidine. Then the slotted block (and slotted gasket if
32　such is used) is rotated horizontally 90°, and each slot
33　well receives another, same or different, reactant so that

　　　　　　　　　　　　position has a predetermined AA. Where the

　　　combinations of dipeptides for a slot block or
　　substrate area plate system. Iterative application of

1   allows for the identification of the single optimal
2   binding peptide from a 64m hexapeptide SPCL.   The
3   substrate areas can be quite small to provide peptides in
4   adequate (picomolar) amounts, e.g., the 1/4" diameter
5   winks.

6   As an illustration of another method of use of the
7   system of this invention, a random sequence of all XXXX-
8   tetrapeptides on Pepsyn-K beads is prepared, and these
9   beads adhered to the substrate areas.   Alternately and
10   preferably, a polymeric film gel (HPMP) may be prepared on
11   a plurality of winks, which are then reacted in a flask
12   (100 ml/100 winks) of an automated peptide synthesizer to
13   produce the random XXXX-tetrapeptide mix. These winks are
14   then pressed into the holes in a wink carrier plate. The
15   wink plate is placed on a border or periphery gasket on a
16   vacuum block, and then overlain with another border gasket
17   and a "window frame" border block and clamped.

18   Then, using the system of this invention, the
19   terminal two Aas can be added thereto in the all-
20   combinations 20x20 array via the rotation of the slotted
21   block assembly.   Alternately, a known dipeptide can be
22   added to the end terminal by two cycles of reaction with
23   the vacuum block, say all VY, i.e., VY at all positions.
24   A reaction cycle is defined as deblocking the prior
25   reaction step AA, and reacting with the next blocked AA.
26   The resulting hexapeptides are screened (probed) by
27   exposure to labeled targets.   This is done in the vacuum
28   block assembly.

29   A deductive process involving iterative resynthesis
30   of successively smaller libraries can be used to
31   successively characterize the resulting screening-active
32   hexapeptide. Alternately, the procedure and apparatus of
33   this invention can work from a defined middle dipeptide
34   with random ends, followed by replacement of each end in
35   sequence with known dipeptides. Likewise 4 or 5 residues
36   may be mixed, or an array of any kind of peptide,
37   including those including one or more non-natural Aas, can
38   be employed on the reusable substrate of this invention.
39   The use of winks receivingly engaged in the support

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1   address area is preferred for diagnostic or drug use
2   applications, as single preselected, known-sequence
3   peptide-containing winks can be prepared in place in the
4   carrier plate, or separately prepared in an automated
5   synthesizer and inserted in the holes in the support in
6   specified address(es).   Likewise, DNA moieties can be
7   bonded to the support, in which case a 16x16 array, or an
8   array of 16-4x4 subarrays, on a single plate is preferred.

9   Advantages of the apparatus and methods of this
10 invention include: Synthesis of defined peptides, portions
11 of which optionally can consist of redundant known or
12 unknown (uncharacterized or non-defined sequence) mixtures
13 which are bonded in micromolar amounts in defined arrays
14 with known addresses so that a physical barrier (e.g. an
15 appropriately apertured member) can permit simultaneous
16 screening.   Another feature is fluorescent or radio-
17 labeled detection of binding, which provides higher
18 sensitivity and is far more suitable for detection of low
19 affinity interactions than the current Selectide or Iterex
20 technology.   The solid array support also permits
21 inference of optional binding elements (e.g. AA sequences)
22 from the spatial position (unique address) rather than
23 requiring chemical determination of sequence.

24   The ASBCLs and ASPCLs constructed by the method and
25 system of this invention are selectively variable at any
26 two or more positions, while redundant (random selection
27 of all combination) at several other positions (say, 3-6)
28 within peptides or biopolymers of a wide range of size and
29 structure.   The system is also useful for screening
30 (probing) by itself, or in conjunction with current
31 methods (such as the Iterex Tea-Bag or Selectide methods),
32 for any two or more AA position sequencing, and can be
33 used for progressive refinement of initially identified

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be functionalized for synthesis of peptides at loadings as
low as about .001 micromoles per cm², usually in the range

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1   loading for HPMP winks.

2   The system also permits simultaneous or sequential
3   synthesis by standard Fmoc or t-Boc-chemistry of
4   identified areas (addresses) of distinct known or non-
5   defined peptides, by attaching the slotted block to the
6   substrate plate for simultaneously performing individual
7   separate couplings in the slot compartments. By
8   transforming the block orientation, arrays of peptides may
9   be synthesized at any two positions within a peptide or
10 biopolymer of any length. The previous or following Aas
11 in the peptide sequence may be uniform across the entire
12 substrate surface, and may be unique or consist of
13 mixtures of one or more peptides of known or
14 uncharacterized composition. Common steps can be carried
15 out in the vacuum block system, and winks loaded with
16 random peptides may be prepared in an automated peptide
17 synthesizer.

18   While the same block is shown used in different
19 orientations, e.g. rotating a slotted block 90°, two
20 dissimilar blocks may be used in the array generating
21 steps, such as a radial slot block (slots extending
22 radially outwardly from a common center) in combination
23 with a block with concentric circular (annular) slots, and
24 the resultant array may be addressed by polar coordinates.

25   The system of the invention permits displacing the
26 label on the target with a natural ligand to insure
27 specificity of the identification. It also permits reuse
28 of the substrate for repeated probing of the surface by
29 alternative proteins i.e. exposure to different targets
30 followed by washing. DMF washing is particularly easy by
31 use of the vacuum block where the wash is removed by
32 aspiration through the vacuum base. Different areas
33 (addresses) may employ the same or different binding
34 materials, e.g. Pepsyn K particles in one area, winks in
35 another, and grafted films in another.

36   Although the method and apparatus shown herein are
37 directed to definition of optimal binding linear
38 hexapeptides, it has great applicability in different
39 formats. Especially where the protein of interest, e.g.

1 cytokine receptor, binds a large ligand, then it is
2 advantageous to insert the library within longer
3 sequences, particularly those which are known to form
4 stable secondary structures, as in loops, beta
5 conformations or alpha helices. In the latter, since the
6 library is then displayed on a cylindrical surface, it is
7 of interest to construct the components 3 or 4 residues
8 apart, the components being separated by helix-forming
9 residues, such as alanine. For libraries based on loop
10 structures, either end may be designated as a Cys residue
11 which can then be coupled together by intramolecual
12 disulfide bridges. Cyclic peptides, especially cyclic
13 hexapeptides and cyclic decapeptides can be constructed on
14 PILOT substrate matrices of this invention, and are
15 especially useful for the relative rigidity of these
16 molecules compared to their linear counterparts. Also,
17 bogus pseudo array checking can be easily done with the
18 system of this invention.

19   The novel PILOT ASBCL's and methods of this invention
20 provide distinct advantages over the numerous alternatives
21 discussed above in the Background to meet the need for
22 developing new pharmaceutically useful compounds. The
23 specificity of the binding may be uniquely established by
24 side-by-side comparative processing of dual plates which
25 are then probed, one with the presence of the natural
26 ligand, the other without, and the two compared.

27   One particular advantage of the invention is that it
28 allows detection by numerous methods, but it is unique in
29 being suitable for detection with radiolabelled
30 derivatives, with autoradiographic and counting methods
31 providing the enhanced sensitivity vital for the detection
32 of relatively low-affinity binding peptides which are
33 present in picomolar amounts within pools containing

      of other non-binding sequences. Use of ^{125}I

37 labeling, arrays are recoverable and can be reprobed
38 numerous times after scintillation counting of individual

1   Another advantage of the invention is that it allows
2   for the use of standard substrate materials (e.g. HPMP
3   winks or Pepsyn K for peptide synthesis, and HPMP winks or
4   controlled pore glass for DNA/RNA synthesis), for
5   synthesis on the plates, or for preassembly by automated
6   synthesizers followed by arraying these for diagnostic
7   applications. In library applications, the unique method
8   of grafting in-situ generated polymers or attaching
9   preformed polymers to functionalized polyolefin surfaces
10   such as winks provides materials far better suited for
11   screening methods than conventional particulate solids.
12   A special virtue is the optical clarity of the HPMP film
13   substrates of this invention, combined with their low
14   intrinsic fluorescence which greatly enhances the
15   sensitivity when used with fluorescent tags. Of even
16   greater importance is that the substrate HPMP films of
17   this invention are formulated to provide excellent
18   penetration of proteins within their bounds, and side-by-
19   side comparisons with prior art methods have shown
20   significantly enhanced sensitivity with use of this
21   invention.

22   This invention is unique in being suitable for
23   construction of libraries containing monomer units of
24   almost any kind, for example, bound together by ether,
25   thioether, ester, amine, phosphate, amide or any such bond
26   establishable by organic chemistry methods.
27   Identification is performed solely through spatial
28   recognition, and does not require sequencing, which is
29   generally impossible with other than natural peptide and
30   DNA units.

31   The PILOT ASBCL's and methods of this invention, are
32   therefore unique, simple, generally applicable and readily
33   duplicated. They provide high sensitivity detection by a
34   variety of tagging procedures.

35   It is important to the application of the PILOT
36   system of this invention as a general library method to
37   equally incorporate amino acids from mixtures of Fmoc-
38   amino acids. We have found that differences in
39   incorporation diminished with increasing concentration,

1   and D-derivatives coupled at rates essentially the same as

2   their L-counterparts.

3   However, rather than use the Rutter-Santi method of

4   employing concentrations based on the coupling constants

5   of the amino acids, we use a non-theoretical, empirical

6   method of determining the molar ratios for equal

7   incorporation of Fmoc-Amino acids from mixtures. These

8   mixtures are used to prepare the random X_n-peptides. We

9   employ 16 AA's to form a standard mixture, Ω. As used

10   herein, Mix • Mix, $(Mix)_2$ or Ω-2 refers to two reaction

11   cycles with the Ω mixture.

12   Based on Example 1 below, the molar ratios described

13   in Table 1 below give substantially equal incorporation on

14   HPMP winks as confirmed by both amino acid analysis and

15   sequencing. Sixteen amino acids are incorporated in our

16   standard mixture Ω. Mixtures may be incorporated at 6 or

17   more positions in a core sequence, and "arrayed" at any

18   other two or more positions. Bogus arrays may be

19   constructed using conventional peptide synthesizers.

20   Study of non-arrayed mixed winks in the presence and

21   absence of natural ligand(s) shows whether a detailed

22   array study will be able to uncover binding sequences.

23   The molar ratios of Table 1 are obtained by coupling, AA's

24   selected with 1.25 equivalents HOBt + 1.5 equivalents

25   PyBOP and 1.5 equivalents 0.3M NMM in DMF after 10 minutes

26   preactivation.

Table I

Molar Ratios For Equal Incorporation of
Fmoc-Amino Acids From Mixtures

Fmoc-Derivative	Molar Ratio	Fmoc-Derivative	Molar Ratio	Fmoc-Derivative	Molar Ratio
L-Nle*	1.00	L-Ala*	0.79	D-Nap*	1.50
L-Leu	1.00	L-Ser(tBu)*	1.50	L-Tyr(Tbu)*	1.70
L-Val*	1.60	L-His(Trt)*	2.10	L-Phe*	1.00
Gly*	0.60	L-Gln(Trt)*	2.20	L-Asp(OtBu)*	1.40
D-Ala*	0.79	L-Pro*	1.15	L-Glu(OtBu)*	1.20

1   DRAWINGS:

2   **Fig. 1** is an isometric view of an assembled PILOT

3   slotted block system apparatus, partly broken away to show

4   the various parts in proper alignment and ready for

5   introduction of AA's for reaction with the substrate areas

6   or moieties thereon;

7   **Figs. 2a and 2b** are section views taken in elevation

8   along line 2-2 of **Fig. 1** showing two alternative

9   structures of a substrate area in detail;

10   **Fig. 3** illustrates using a combination of two

11   different blocks, one radial and one with concentric

12   annular slots, with a circular support plate to produce a

13   circular array;

14   **Fig. 4** is a section view of the concentric annular

15   block taken along line 4-4 of **Fig. 3**;

16   **Fig. 5** is an isometric view of the peripheral frame

17   system of this invention for functionalizing plates prior

18   to condensing biopolymers thereon;

19   **Fig. 6** shows in exploded isometric view the presently

20   preferred embodiment of the slotted block assembly of **Fig.**

21   **1** employing a vacuum disc-holding array plate for the

22   array preparation steps;

23   **Fig. 7** shows in exploded isometric view the use of

24   the vacuum plate on the vacuum base for common peptide

25   synthesis and probing steps;

26   **Fig. 8A** is an enlarged vertical section view through

27   one hole of the vacuum block with a single vacuum draw and

28   drain hole below the sintered disc "wink" in place in an

29   array hole;

30   **Fig. 8B** is a vertical section-view through an

31   alternate embodiment of the vacuum plate with multiple

32   vacuum/drain holes;   **Fig. 9** shows in isometric a

33   slotted gasket used above the plates of **Figs. 1, 6** and **7**

34   in place of the multi-hole array gasket 10 of **Figs 1** and

35   **5**;

36   **Fig. 10** is a graph of the percent amino acid vs.

37   molar percent incorporation in equi-molar mixtures to

38   prepare the Ω mixtures used herein; and

39   **Fig. 11** shows the results of the ^{35}S streptavidin

1   array test.

2

3   **DETAILED DESCRIPTION OF THE BEST MODE OF THE INVENTION:**

4   The following detailed description illustrates the

5   invention by way of example, not by way of limitation of

6   the principles of the invention. This description will

7   clearly enable one skilled in the art to make and use the

8   invention, and describes several embodiments, adaptations,

9   variations, alternatives and uses of the invention,

10   including what we presently believe is the best mode of

11   carrying out the invention.

12   Referring to **Fig. 1** the slotted block system of this

13   invention 1 includes a square base 2 which has or receives

14   orienting members, such as a plurality of guide and

15   securing rods 3 with wing nuts 4. The square base 2

16   receives a square substrate support plate 5 which includes

17   an array of areas 6. There are three basic variations in

18   the substrates: in situ beads, film, or discs as shown in

19   **Figs. 2a, 2b** and **8a/8b**, repectively.

20   In a first, embodiment shown in **Fig. 2a**, each area 6

21   includes substrate 7 (in this case beads) secured in

22   depression 8 by a suitable glue 9. The second embodiment

23   of **Fig. 2b** shows a grafted polymeric film as the substrate

24   7 in depression 8. **Figs. 8a** and **8b** show the porous

25   sintered polyolefin HPMP coated disc, which is the

26   presently preferred embodiment.

27   Overlying the substrate support plate 5 is a square

28   gasket 10, preferably a sheet of chemically inert

29   elastomeric material (e.g., Viton or silicon rubber),

30   having an array of holes 11 therethrough which are the

31   same size as and in alignment with the substrate areas 6.

32   See **Figs. 1, 2a, 2b, 3, 6** and **9**. The gasket functions to

33   prevent leakage between individual substrate areas 6 or

34   discs 50. An alternative slotted gasket 10a, shown in

     gasket 10a may be glued to the underside of the slotted

     block 12. Still another alternative is to provide O-

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1   surrounding each area. Or a groove can be provided on the
2   underside of each slotted block surrounding each slot,
3   which groove receives a round (in cross-section) seal
4   strip.

5   A square slotted block 12, having holes 13
6   therethrough to receive the guide/securing rods 3 is
7   placed over the gasket 10. This block 12 includes a
8   plurality of slots 14, 15, 16 etc. therein, which are
9   aligned with and extend a full row width of the substrate
10   areas 6. The slots may be identified, as by the alpha
11   designations A,B,C etc. shown. Note in the substrate
12   support plate 5, additional corresponding rows L,M,N...
13   are shown. In a typical block 12, there will be some 4 to
14   10 and as many as 400 such slots. Fastening the wingnuts
15   4 secures the assembly together in the proper orientation
16   and prevents leakage between adjacent slots and substrate
17   areas when individual reaction solutions are placed in the
18   wells formed by the slots.   **Fig.** 6, the presently
19   preferred embodiment in which the wink carrier plate 5' is
20   used, employs the same top gasket 10, but uses a hole-less
21   bottom gasket 49 to seal between adjacent holes.

22   Continuing with **Fig. 1**, after reaction, removal of
23   solution, washing and deprotecting, the square block 12 is
24   rotated by 90° and selected solutions are introduced in
25   the chosen slots, to produce a known array of dipeptide
26   sequences.   This cycle can be carried out with the
27   apparatus of **Fig. 6** as well.

28   **Fig. 3** shows an important variation involving a round
29   substrate support plate 20 having a concentric/radial
30   array 21 of discrete substrate areas or holes 6. A gasket
31   22 also includes a corresponding concentric/radial array
32   of holes 23. In this embodiment, two slotted plates may
33   be employed sequentially: slotted radial plate 24, and
34   concentric slotted plate 25, in either sequence, 24, 25 or
35   25, 24. There may be fewer radial slots 26 than the
36   radial array of holes 23 or substrate areas 21, in which
37   case the block 24 may be turned between application of
38   reactants (e.g., AA solutions). The inner concentric
39   segments 27, 28, 29 etc. may be secured in spaced

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1   relationship by horizontal rods 30, 31, which are spaced
2   above the bottom 32 of block 25 to insure access of
3   solution to all relevant substrate areas. In **Fig. 2** the
4   base plate and pins are omitted for clarity.

5   **Fig. 4** shows in vertical cross section the
6   construction of the concentric slotted plate 25 in which
7   outer ring 27 is spaced from inner core 34 by rod 31. A
8   series of tubular spacers 35, 36, 37, 38 on rod 31 space
9   the concentric intermediate rings 28, 29 from core 34 to
10 provide concentric annular slots. The rod 31 may be
11 countersunk in bore 39.

12   **Figs. 5** and **7** show a border frame assembly for
13 functionalizing the substrate areas 8 on support plate 5,
14 e.g., with common Aas, or mixtures of Aas, and for
15 deblocking, washing, probing (screening) and addition of
16 spacer arm derivatives. An edge gasket 40 is placed on
17 the substrate plate 5, then a border frame 41 is placed
18 thereover and secured with clamps 42, 43. This provides
19 a central well 45 for the entire array for simultaneously
20 receiving the appropriate solutions for the
21 functionalizing chemistry.

22   **Fig. 7** shows a border frame assembly in association
23 with a vacuum base 46. In this embodiment the wink
24 carrier plate 5' is sandwiched between two identical edge
25 or periphery gaskets 40a, 40b and placed on vacuum base 46
26 having a trough-shaped interior cavity 47 and an
27 aspiration outlet tube 48. The border frame 41 is placed
28 on top of gasket 40a and the entire assembly clamped
29 together, by rods 3 through holes 13 which are secured by
30 wingnuts 4. This is the preferred assembly for common
31 steps, e.g., of adding the previously determined or
32 selected di, tetra-, hexa-, etc, peptides and for
33 deprotecting, washing and probing. The tube 48 is
34 connected to a vacuum source, such as a water aspirator,

    trough 47.

36   **Figs. 8a** and **8b** are enlarged vertical section views

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holes 51 bored partway through carrier plate 5'. A slight tapered shoulder 52 permits drainage via hole 53 when suction is applied from below.   The wink diameter is typically 1/4", and hole 53 is 1/8".   Pressure P from finger 54 is sufficient to press-fit the winks 50 into holes 5'.   A dowel or Q-tip 55 inserted in hole 53 is sufficient to remove the wink. **Fig. 8b** shows a variation in which multiple drain holes 56, 57, 58 etc. may be employed. A smaller dowel or comb-like pusher may be used to remove the winks by insertion through holes 56-58.

The wink carrier plate 5' securely holds porous polyolefin discs 50 throughout the course of the array and probing.   Dextrainized winks are prepared as described above.   Common unarrayed sequences are assembled on the winks using commercial synthesizers.   They are then mounted in plate 5' as shown in **Fig. 8a**.   To directly perform an array the plate is mounted in the slotted block apparatus of **Fig. 6** with a solid viton gasket 49 between it and the base plate, and a regular holed gasket 10 placed between the plate 5'and the slotted block 12. Coupling of one dimension of the array is then performed. The plate is then transferred to and secured in the vacuum base apparatus of **Fig. 7**, along with edge gaskets 40a and 40b, and the window frame block 41, and washed with DMF (e.g., shaken with DMF for 30 seconds) then the wash is removed by aspiration through the base 46.   This washing is extremely efficient in comparison to the bath technique of **Fig. 1**.   This is followed by Fmoc removal steps (deprotection), and more washings performed analogously. The second dimension of the array may then be performed, or common sequences introduced, as required.   Following assembly of the array, the completed plate mounted in the vacuum block of **Fig. 7** is treated with TFA + scavengers to remove side-chain protection.   Following washing with methanol, DMF and water, the plate is then washed and thoroughly equilibrated with assay buffer.   The radio-labelled protein is then introduced, and the plate probed as appropriate (10 minutes to 2 hours).   A parallel plate produced in a second apparatus of **Fig. 7** can be used to

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1   simultaneously determine the specificity of binding (i.e.,
2   by addition of natural ligand).   After incubation, the
3   plate 5' is suction washed rapidly with 4 additions of
4   buffer (20 Ml), removing unbound label.   Binding may be
5   determined by autoradiography. With weaker isotopes, the
6   individual winks may be punched into scintillation vials
7   and counted. This is a simple and quantitative procedure.
8   Of great importance, we have found that, in a variety of
9   cases, the winks can be recovered, the radioactivity
10   displaced, and the winks reused for similar or different
11   assay purposes.   The reusability of the system is one of
12   its key attributes.
13   The   following   example   protocols   describe   the
14   sequential steps of the method:
15
16   **Example   1 - Determination of AA Mixture Proportions for**
17   **Equimolar Incorporation on Substrates - Empirical**
18   **Method:**
19   The component amino acid (Fmoc-X-OH,1 equivalent) is
20   mixed with Fmoc-Nle-OH (1 equivalent), then dissolved and
21   activated   by   the   addition   of   PyBOP,   HOBt   and   NMM
22   solutions. After 10 minutes the mixture is added to Nva-
23   PAL-Pepsyn K support (5 mg). After 2 hours the support is
24   washed with DMF repeatedly, treated with 30% piperidine in
25   DMF (to remove incorporated Fmoc- groups), washed with
26   DMF, and methanol, then treated with TFA/water (95:5) for
27   2 hours. The TFA solution is expelled into a vial, a known
28   proportion of it dried down in vacuo, and the resultant
29   mixed dipeptides X-Nva and Nle-Nva are hydrolysed with 6M
30   Hcl at 150 degrees for 1 hour. The relative incorporations
31   of X and Nva are then determined by amino acid analysis.
32   A graph is constructed plotting molar % X (in this
33   initial round X is 50%) against molar percentage
34   incorporated; and the curve which results when using

Nle.   The molar percent incorporation of an individual
18   amino acid is the amount of the amino acid divided by the

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1   AA/(AA + NLE + NOR) = % incorporation). As shown in Fig.
2   10 the molar percent incorporation is then plotted against
3   the percent amino acid in the mixture. The zero and 100%
4   data points are also included. Draw a line from the 50
5   percent point on the y-axis to the curve and then drop a
6   line from that point to the x-axis and determine the
7   percent molar incorporation necessary to obtain a 50
8   percent amino acid mix. In this example, the first
9   evaluation was made with 50% Asparagine and 50%
10   Norleucine. This gave a mixture of approximately 30%
11   Asparagine and 70% Norleucine. A refined evaluation was
12   made with 67% Asparagine which gave a mix of 45%
13   Asparagine/55% Norleucine. The final value was determined
14   to be 71% Aspargine to achieve a 50% mix.

15   The process is repeated using the predicted molar
16   percent X to confirm and, if necessary, iteratively refine
17   the molar percent. This method has been applied to all 20
18   natural L-amino acids, most D-amino acids, and several
19   unnatural amino acids, such as beta-alanine and 2-
20   napthylalanine.

21   For any desired library mixture, the amino acids are
22   selected, mixed in the correct ratios, activated, coupled
23   to the support, and the equal incorporation confirmed by
24   analysis. For the 10 amino acid library the subject of
25   these examples the following recipe gives equal
26   incorporation: Fmoc-L-Nle-OH 0.188g; Fmoc-L-His(Trt)-OH
27   0.73g; Fmoc-L-Pro-OH 0.24g; Fmoc-L-Gln(Trt)-OH 0.797g;
28   Fmoc-L-Tyr(Tbu)-OH 0.398g; Fmoc-Gly-OH 0.093g; Fmoc-L-Phe-
29   OH 0.206g; Fmoc-L-Arg(Pmc)-OH 1.25g; Fmoc-L-Glu(OtBu)-OH
30   0.288g; and Fmoc-D-Ala-OH 0.130g. To these mixed amino
31   acids were added HOBt 1.614g, and the entire solids
32   totally dissolved in DMF and made up to a volume of 40 Ml.
33   For coupling, 10 Ml of this solution, called MIX solution,
34   is added to 1.71g PyBOP reagent, mixed, 0.35 Ml of N-
35   methylmorpholine is added, remixed and left for 10
36   minutes. This solution is adequate to completely cover and
37   react a single 10 x 10 plate giving equal incorporation.
38   Table I and its related description above show the
39   quantitative amounts in this example converted to molar

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1 ratios.

2

3 **Example 2 - Preparation of A "Beaded" Plate (Support Plate**
4 **With Array of Bead-Type Substrate Areas):**
5   The sequence BAla-Bala-Bala-Nle-Bala-Nle-Bala-Bala
6 was assembled on 0.2 mmol/g Pepsyn-K (Millipore)
7 functionalized by treatment with ethylenediamine using a
8 Milligen/Biosearch model 9600 peptide synthesizer using
9 standard BOP + HOBt coupling protocols. Ace Hardware Hot
10 Melt adhesive was cut into thin sections and melted at as
11 low a temperature as possible on a flat PTFE sheet to
12 produce a thin sheet (in a range of from about .2 to about
13 1.0mm thick) of hot melt adhesive (HMA sheet). The PTFE
14 sheet was removed from the heat, and dry, peptide-bearing
15 Pepsyn-K beads were sprinkled over the melted glue surface
16 and gently patted down. After several hours of cooling,
17 excess beads were removed and the glue sheet lifted off
18 the PTFE sheet, then punched into appropriate circles
19 (e.g. 1-10mm dia) with a standard hole punch. The
20 resultant discs were then attached to an array of shallow,
21 dished wells in a polyethylene sheet using a Black and
22 Decker Thermogrip glue gun adding a dab of glue in the
23 well and pushing the discs down firmly. The discs can be
24 reinforced with polypropylene or metal mesh. Typically,
25 each patch bears 5 mg of beads, having 1 micromole of
26 spacer arm linked Pepsyn-K. The top right hand corner of
27 every plate is notched or drilled as a reference to
28 prevent the plate being incorrectly aligned at any step.

29

30 **Example 3 -   A Preparation of A Polymeric "Film" Plate:**
31   A 10mm thick plate of linear high density
32 polyethylene is floated in a water bath at 70°C and treated
33 with 5M chromium trioxide in 5.3M sulfuric acid for two
34 hours. The plate is washed with water many times then

    to acid chlorides by treatment with ... thionyl chloride
3? in chloroform for two hours. The plate is rapidly washed

1   nitrogen and used immediately.   This acid chloride
2   functionalized plate can be derivatized by a variety of
3   reagents to introduce many functionalities.   Linear
4   p o l y m e r s ,   s u c h   a s   polyethylenimine,
5   poly(amino)functionalized polyethylene glycols, and
6   saccharide may be added to the plates by conventional
7   chemistries.   For example, for introduction of acryloyl
8   groups, the plate may be treated with either a solution of
9   N-(3-aminopropyl) methacrylamide hydrochloride and
10   triethylamine in DMF, or it may be treated with neat
11   (undiluted) diaminopropane for two hours, followed by
12   washing thoroughly with water, then methanol, then ether,
13   and then treated with a solution of acryloyl chloride and
14   disopropylethyl amine in THF.   The plate is washed well
15   consecutively with methanol and ether, and dried under
16   vacuum.   The plate is now ready for grafting or casting of
17   a gel film thereon as the substrate in the specific
18   address areas.

19   The gels which may be cast into the wells of the
20   substrate plate or grafted to the acryloyl groups on the
21   surface of a film plate can have a variety of
22   concentrations, cross-linking levels, functional linkers
23   and amino linker loading.   To prepare a typical gel, under
24   nitrogen, a 5 ml portion of deoxygenated water (under
25   vacuum for 20 minutes) is added to 18.5 mg of
26   bisacryloyldiaminohexane, 295 mg of dimethylacrylamide,
27   and 186 mg of the monoacrylamide of 1,6 diaminohexane
28   hydrochloride.   This is filtered onto 15 mg of ammonium
29   persulfate and treated with 30 uL of Ph 6 TEMED solution
30   in water.   In a glove bag under nitrogen, the monomer
31   solution is rapidly transferred to each well of the plate.
32   The plate is sealed in a plastic bag with an open beaker
33   of deoxygenated water and allowed to gel.   After curing
34   over night the plate is washed with water and soaked in 1N
35   sodium hydroxide for 2 hours.   Two water washes of 15
36   minutes each followed by at least four washes in DMF give
37   a plate which is ready for peptide synthesis.
38
39   Example 4 -   Spacer Arm Derivatization of The Film:

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1   Optionally a gel film plate of the type in Example 3
2   may have a tetrapeptide spacer attached to the substrate.
3   A plate prepared as in Example 3 had Fmoc-beta-alanine
4   (Bala) coupled to it (standard PyBOP + HOBt/NMM procedure,
5   2 hours). Following thorough DMF washes, the plate was
6   treated with 30% piperidine in DMF (1, 45 minutes). The
7   plate was washed 2 times with DMF, and the piperidine
8   treatments and subsequent washes were pooled and read
9   spectrophotometrically at 301 nm to determine the Fmoc-
10   loading (in this example 2 micromoles per substrate area).
11   Three more coupling cycles were then performed adding
12   Fmoc-epsilon-aminocaproic acid twice, then beta-alanine
13   again to give the final Bala-Aca-Bala spacer arm film
14   plate as used in the preferred embodiment for ASBCL or
15   ASPCL libraries.

16

17   **Example 5 - Construction of A 10x10 Hexapeptide ASPCL**
18   **Plate:**

19   The method of this invention allows the construction
20   of arrays of sequences at any 2 sequence positions within
21   peptides of any reasonable size with several positions
22   being incorporated as mixtures. The preferred embodiment
23   of the method is to prepare hexapeptides with the central
24   2 AA's arrayed, the other 4 positions redundantly mixed,
25   and the final sequence AA is N-acetylated. For film
26   plates it is preferred to add a spacer arm peptide to the
27   film prior to construction of the library, and with beaded
28   plates an octapeptide spacer is attached prior to
29   embedding in the glue. The following sequence of
30   operations is followed to prepare a Hexapeptide ASPCL with
31   a known central (3,4) AA sequence:
32   i)   Apply an edge gasket and a border-frame spacer
33   on the substrate plate (see Fig. 5) to make a
              flat "dish" type reactor. As an alternative to

              or wing nuts and standard bolts and washers to
              hold the parts together;

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iii)　Couple 10 Ml preactivated MIX solution made as per Example 1 for 2 hours, while covering plate with foil tent;

iv)　Wash with DMF 3x;

v)　Deblock with 30% piperidine in DMF 1 min, 10 min;

vi)　Wash with DMF 5x;

vii)　Repeat steps iii) to vi);

viii)　Dismount edge gasket and frame spacer, and mount slotted block assembly with 100 hole gasket to base plate as in Fig. 1 with slots in a first, horizontal orientation (L TO R when facing the assembly). DMF solvent is placed in alternate wells and the dry wells observed carefully to ensure no leakage. Prepare in vials the following amino acids: 1. Fmoc-L-Nle-OH 0.14g; 2. Fmoc-L-His(Trt)-OH 0.25g; 3. Fmoc-L-Pro-OH 0.135g; 4. Fmoc-L-Gln(Trt)-OH 0.244g; 5. Fmoc-Tyr(Tbu)-OH 0.183g, 6. Fmoc-Gly-OH 0.116g; 7. Fmoc-L-Phe-OH 0.154g; 8. Fmoc-L-Arg(Pmc)-OH 0.265g; 9. Fmoc-L-Glu(OtBu)-OH 0.17g; 10. Fmoc-D-Ala-OH 0.124g. To each of these vials add and mix PyBOP 0.27g and HOBt 0.06g, and 2 Ml of 0.3M N-methylmorpholine in DMF. Add each to a designated horizontal slot: 1 to top slot; 2 to next slot, etc.;

ix)　Maintain at room temperature for 2 hours to complete coupling;

x)　Disassemble and remount with edge-gasket and border-frame spacer;

xi)　Wash with DMF 3x;

xii)　Deprotect with 30% piperidine in DMF 1 minute, 10 minutes;

xiii)　Wash with DMF 5x;

xiv)　Mount slot block with slots rotated 90°, i.e., in a vertical orientation, and repeat coupling as described in viii) except 1 is coupled to the left hand slot, 2 to the next slot, etc.;

1   xv) Disassemble and remount with edge-gasket and
2   border-frame spacer;
3   xvi) Remove Fmoc group and perform 2 cycles of
4   mixture incorporation as described in i) to
5   vii);
6   xvii) Remove Fmoc-group and wash with DMF 5x;
7   xviii) Acetylate with 0.3M acetic anhydride + 0.3M
8   HOBt in DMF (10 Ml) for 1 hour;
9   xix) Wash DMF 5x, Methanol 5x;
10   xx)   Treat with 95:5 TFA/water for 2 hours;
11   xxi) Wash Methanol 5x, aqueous buffer 5x. Store in
12   sealed bag at 4 degrees prior to probing to
13   screen a target.

14 Numbering from the carboxy terminus, the resulting
15 hexapeptides are characterized as $XX-A_4$, A_3-XX with the A_3
16 and A_4 known sequence being uniquely addressable. That is,
17 the central dipeptide is known from its unique address by
18 use of the slotted block, the hexapeptide at address 001
19 being XX-D-Ala-L-Nle-XX, at address 002 being XX-L-Glu-L-
20 Nle-XX, etc., to address 100 being XX-L-Nle-D-Ala-XX.
21

22 **Example 6 - Construction of A 10x10 Hexapeptide ASPCL on**
23 **a Gel Film Plate**
24 Instead of a bead plate, a gel film plate as in
25 Examples 3 and 4 may be similarly employed to construct an
26 ASPCL library by the process of Example 5. This gel film
27 ASPCL is used to screen, see Example 7 below.
28

29 **Example 7 - Determination of Binding Elements in the**
30 **Interaction of Streptavidin with Peptides**
31 A film library plate of Examples 3 and 4 was
32 constructed similarly to Example 6 with the selection of
33 10 amino acids as indicated. ^{125}I labelled streptavidin
34 was prepared and purified by standard procedures; a

35 being removed by dialysis. Firstly, the iodinated protein
36 was incubated with the plate overnight in a phosphate

1   serum albumin (1mg/Ml).   The plate was washed with the
2   buffer 3 times, placed on a standard laboratory X-ray film
3   with an enhancer plate and exposed overnight.   The
4   developed film shows strong affinity in specific address
5   locations corresponding to 2 central dipeptides HP and RR.
6   The plate was then washed repeatedly with 6M guanidine
7   hydrochloride, and buffer medium, then reincubated with
8   the fluorescent AMCA-streptavidin overnight.   After
9   washing the plate was irradiated with long wave length uv
10 light and visible confirmation obtained of the previously
11 deduced binding elements.   Subsequent iterations as
12 described above further defines the active dipeptides at
13 each end for complete hexapeptide characterization.
14
15 **Example 8 -   Array Test for ^{35}S Streptavidin Using HPMP**
16 **   Winks in a Carrier Plate**
17   This example details the use of the HPMP winks in the
18 carrier plate 5' of **Fig. 8a** with the slotted block system
19 of **Fig. 6** and vacuum base system of **Fig. 7**.
20   70 dextran functionalized winks were placed in the
21 reaction vessel of a Millipore Model 9600 peptide
22 synthesizer. A mixture of Fmoc-protected amino acids was
23 made by carefully weighing the individual components
24 according to the following list, followed by intimately
25 mixing them in a pestle and mortar: L-NorLeu, 0.093g; L-
26 His(Trt), 0.341 g; L-Pro 0.10g; L-Gln(Trt), 0.351g; L-
27 Tyr(Tbu), 0.203g; Gly, 0.046g; L-Phe, 0.102g; L-Arg(Pmc),
28 0.52g; L-Glu(OtBu), 0.132g; L-Ala, 0.064g; D-Ala, 0.064g;
29 L-Asp(OtBu), 0.150g; L-Val, 0.131g; L-Ser(Tbu), 0.149g; D-
30 (2-napthyl)Ala, 0.153g; L-Lys(Tboc), 0.168g.   Four
31 individual aliquots of the mixture (0.461g) were placed
32 along with PyBOP (0.82g) and HOBt(0.2g) in each of the
33 first 4 reservoirs of the instrument, and synthesis
34 performed using standard Fmoc 4 hour coupling programs
35 with 10 minute preactivation.
36   Following synthesis, 64 of the product winks, now
37 bearing tetrapeptide mixtures of all possible
38 combinations, were placed in an 8x8 array in the standard
39 10x10 plate with blank winks occupying peripheral

1   positions. The plate was marked in its top right hand
2   corner, washed with DMF several times, then placed in the
3   slotted block system of **Fig. 6** with a solid viton gasket
4   between it and the base plate. The standard 100 hole top
5   gasket was then positioned, followed by the slotted block
6   in a vertical orientation. The slots were tested to make
7   sure no leakage was occurring. For array coupling, pairs
8   of amino acids were added to the 8 appropriate slots as
9   follows (each pair also had added 0.80g PyBOP and 0.2 g
10   HOBt, activation was with 5 Ml of 0.3M NMM in DMF): - Slot
11   2, Tyr(Tbu) 0.29g + Phe 0.143g; Slot 3, Asp(OtBu) 0.22g +
12   Glu(OtBu) 0.195g; Slot 4, Arg(Pmc) 0.457g + Lys(Tboc)
13   0.145g; Slot 5, Nle 0.134g + Val 0.21g, Slot 6, Gly 0.128g
14   + D-Ala 0.177g; Slot 7, Ala 0.109g + Ser(Tbu) 0.249g; Slot
15   8, His(Trt) 0.29g + Gln(Trt) 0.312g; Slot 9, Pro 0.146g +
16   DNapAla 0.25g. The apparatus was left to gently shake
17   overnight to insure coupling.

18   The apparatus was disassembled, the plate 5' placed
19   in the vacuum block assembly of **Fig. 7**, washed with DMF
20   (6x), deblocked with 30% piperidine in DMF for 10 minutes,
21   washed DMF (6x). Plate 5' was placed back in the slotted
22   block system of **Fig. 6** with the slotted block being turned
23   90°. An identical array coupling was performed except
24   that the slots were now oriented in a horizontal manner.
25   After a 4 hour coupling, the plate 5' was transferred back
26   to the vacuum base apparatus of **Fig. 7**, washed with DMF
27   (6x), deblocked with 30% piperidine in DMF (10 minutes),
28   then washed with DMF (6x) and methanol (6x). The plate 5'
29   carrying the winks was then treated to remove all side-
30   chain protecting groups for 4 hours with 90% TFA, 5%
31   anisole, 2.5% water, 2.5% dimethyl sulfide. Subsequently
32   it was washed 6 times with methanol, then DMF, water,
33   methanol and water. It was then equilibrated overnight in
     ... ... buffer consisting of phosphate buffered saline

     ....

   To probe the plate, fresh buffer was added ... M..,
   and 100 microliters of standard Amersham ³⁵S labeled
   ....

1   gently for 2 hours, then the supernatant sucked out via
2   the vacuum base. The plate was then washed rapidly whilst
3   rocking with 2 x 20 Ml of assay buffer, then 2x 20 Ml of
4   water. The plate was then separated from the apparatus,
5   inverted, and individual winks poked (**Fig. 8a**) out into
6   clearly   labeled   corresponding   scintillation   vials
7   containing 0.5 Ml of 0.1M Hcl. These vials were shaken
8   for   1   hour   to   displace   bound   activity.   Standard
9   scitillation cocktail (10 Ml) was added, then each vial
10   counted for 5 minutes on a Beckman beta-counter.

11   Results according to array location are depicted in
12   **Fig.   11.**   Surprisingly,   many   areas   have   absorbed
13   radioactivity. Four main peaks were selected, and the 16
14   possible dipeptides (GF, GY, DAY, dAF, NleG, NleDAla, VG,
15   VdA, NleY, NleF, VY, VF, NleNle, NleV, VNle, VV were
16   produced on $(Mix)_4$ winks with the aid of a modified
17   multiple   peptide   synthesizer.   Following   side-cahin
18   deprotection and probing, VY and VF showed maximal binding
19   with >90% of absorbed counts being displaced with biotin.

20   A second array series was then performed. $(Mix)_2$
21   winks were made and mounted in the plate. The array steps
22   were then performed in the central 2 positions of the
23   hexapeptide.   First Y, then V were added to complete the
24   process. Deprotection, probing and synthesis of possible
25   selections found VYGF and VYHP as strong binders.

26   A third array series was then performed with the C-
27   terminal 2 positions arrayed and VYGF appended thereto.
28   Probing showed VYGFRQ as the best combination. Following
29   up a VYHPQ lead, we found VYHPQF and VYHPQV to be good
30   binders, slightly better than HPQFVbA, and our own best
31   sequence HPQVFV.   To test whether the two series of
32   peptides   overlapped   at   the   biotin   binding   site   of
33   streptavidin, a combination nona-peptide, HPQVYGFRQ, was
34   made and found to be a much stronger binder by both
35   BIAcore and PILOT comparison.

36   This example illustrates the true potential of the
37   PILOT method for drug discovery.   Its high sensitivity,
38   utilizing arrays prepared by the simple system of this
39   invention with optimal display chemistry of the HPMP

-33-

1   winks, allows the identification of weak binding elements,
2   which when combined and properly oriented permit advanced
3   pattern recognition for mapping of receiptors to yield new
4   highly active drug candidates and high affinity super-
5   binding compounds.

6   It should be understood that various modifications
7   within the scope of this invention can be made by one of
8   ordinary skill in the art without departing from the
9   spirit thereof. We therefore wish our invention to be
10 defined by the scope of the appended claims in view of the
11 specification as broadly as the prior art will permit.

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CLAIMS

We Claim:

1.   A biological screening assembly comprising in operative combination:

a)   an inert support plate having defined thereon an array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

b)   at least one biopolymer-retaining substrate material secured to each of said addressable areas; and

c)   said substrate material on at least some of said areas of said array is selected from the group consisting essentially of a polymer disc, a polymer film, aparticulate material, and combinations thereof, and said substrate material is amino-functionalized for assembly of biopolymer chains thereon.

2.   A biological screening assembly as in claim 1 wherein:

a)   said polymer film on at least some of said areas of said array is selected from the group consisting essentially of a film polymerized in situ, at least partly preformed polymer, and combinations thereof;

b)   said plate areas include active groups for chemical bonding with components forming said films; and

c)   said substrate includes at least one functionalized spacer arm derivative in at least some of said areas.

3.   A biological screening assembly as in claims 1 or 2 wherein:

a)   said plate is a polyolefin;

b)   said film is a polymer having low molar percentage of crosslinking covalently grafted to said active groups in said areas to form said biopolymer-retaining substrate; and

c)   said spacer arm is amino-functionalized.

4.   A biological screening system comprising in

operative combination:

a) an inert support plate having defined thereon an array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

b) at least one biopolymer-retaining substrate material secured to each of said addressable areas.

c) a barrier gasket sheet overlying said support plate having an array of holes therethrough aligned with said array of areas for access to said substrate areas; and

d) an apertured block disposed on said gasket having a plurality of slots each oriented to provide a common well for simultaneous access to a plurality of said substrate areas in said array.

5. A biological screening system as in claim 4 wherein:

a) said array is selected from a rectangular N x N aray wherein N rages from 2 to 400;

b) said slotted block is selected from a corresponding block having N linear slots positioned to provide access to all the substrate areas of said plate; and

c) said block is orientable on said array in at least two rotationally different positions with respect to each other to permit construction of defined sequences from chemical components placed in said wells.

6. A biological screening system as in claims 4 or 5 wherein:

a) said block includes means for identifying the orientation of said slots to said substrate array to

claims 1- wherein:

a) at least some of said addressable areas

holes in said plate; and

        b)   said substrate material on said discs is a hydrophilic polar multi-functionalized polymer film.

        8.   A biological screening system comprising in operative combination:

        a)   an inert support plate having defined thereon on array of discreet, individually addressable areas for receiving a biopolymer-retaining substrate material;

        b)   at least one biopolymer-retaining substrate material secured to each of said addressable areas;

        c)   at least some of said addressable areas comprise porous polyolefin-discs removably received in holes in said plate, and said substrate material on said discs is a hydrophilic polar multi-functionalized polymer film;

        d)   a border block overlying said support plate;

        e)   a vacuum base underlying said plate; and

        f)   means to seal said plate between said border block and vacuum plate to permit withdrawal of solution applied on said plate through said porous disc and said plate holes.

        9.   A method of preparing chemical polymer sequences on a continuous support substrate comprising the steps of:

        a)   superimposing on a substrate a first block having a first top and a first bottom and a pulurality of first slots disposed to extend vertically between said first top and bottom;

        b)   adding a first pulrality of solution of protected monomers to each of the first slots, wherein the solution in each slot comprises a different known protected monomer;

        c)   reacting the protected monomer solutions with the substrate to attach the protected monomer to the substrate in at least selected areas within the slot and form reacted substrate areas;

d)　　deprotecting at least some of the reacted substrate areas;

e)　　superimposing on the reacted substrate areas a second block having a second top and a second bottom and a plurality of second slots disposed to extend vertically between said second top and bottom, said second block being oriented with its plurality of second slots forming an orthogonal set with said plurality of first slots;

f)　　adding a second plurality of solutions of protected monomers to each of the second slots, wherein the solution in each slot comprises a different known protected monomer; and

g)　　reacting the second protected monomer solutions with the reacted substrate areas to attach the second protected monomer to the reacted substrate areas to form an array of polymer sequences having known monomers in known positions in said sequences at known address locations in said array.

10.　The method of claim 9 wherein:

a)　　said plurality of first slots is a plurality of linear parallel slots;

b)　　said plurality of second slots is a plurality of linear parallel slots; and

c)　　step (e) is performed by rotating said first block 90°.

11.　The method of claims 9 or 10 wherein:

a) other moieties may be interposed and reacted with the deprotected substrate of step (d) before reacting the second plurality of solutions of steps (e), (f) and (g)';

　　　　　　　　the monomers are selected from the group

monomers, protected monosaccharide units, and mixtures thereof;

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monomers are attached in said polymer sequence in any predetermined order selected from sequential to each other to separated by other moieties.

12. The method of any of claims 9-11 wherein:

a) the positions in said polymer sequence other than occupied by said known monomers are selected from the group consisting essentially of random equimolar monomers and polymers; and

b) the monomers are selected from the group consisting essentially of protected L-, D- and non-natural amino acids, protected DNA monomers, protected RNA monomers, protected monosaccharide units, and mixtures thereof.

13. The method of any of claims 9-12 wherein:

a) the positions in said polymer sequence other than occupied by said known amino acids are selected from the group consisting essentially of random equimolar mixed amino acids, dipeptides, tripeptides and polypeptides.

14. The method of any of claims 9-13 which includes the added steps of:

a) screening said array of polymer sequences having two known monomers in any known position against a preselected target to locate by address in said array on said substrate at least one polymer sequence having a desired property;

b) determining the known monomer sequence by reference to the address in said array on said substrate;

c) repeating the steps of reacting protected known monomers at determined positions in said polymer sequence different than the positions of said first and second known monomers to form a second array;

d) repeating said screening against said preselected target;

e) repeating said determination of the known monomers sequence; and

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f)   repeating steps c, d and e at least one additional time to fully characterize the polymer sequence active with respect to said target.

20

15.   The method of any of claims 9-14 wherein:

a)   said monomers are L-, D- and non-natural amino acids; and

b)   said polymer sequence is a hexapeptide forming an ASPCL.

5

16.   A method of diagnosis for biologically active target molecules comprising the steps of:

a)   providing an ASBCL plate having an addressable array of known target active sequences thereon;

5

b)   exposing said ASBCL plate to at least one solution expected to contain at least one target; and

c)   identifying any target in said solution by binding at an address on said array that is site specific for said target.

10

17.   A method of diagnosis as in claim 16 wherein:

a)   said ASBCL plate array includes at least some peptide sequences thereon.

18.   A method of diagnosis as in claims 16 or 17 wherein:

a)   said ASBCL is an ASPCL.
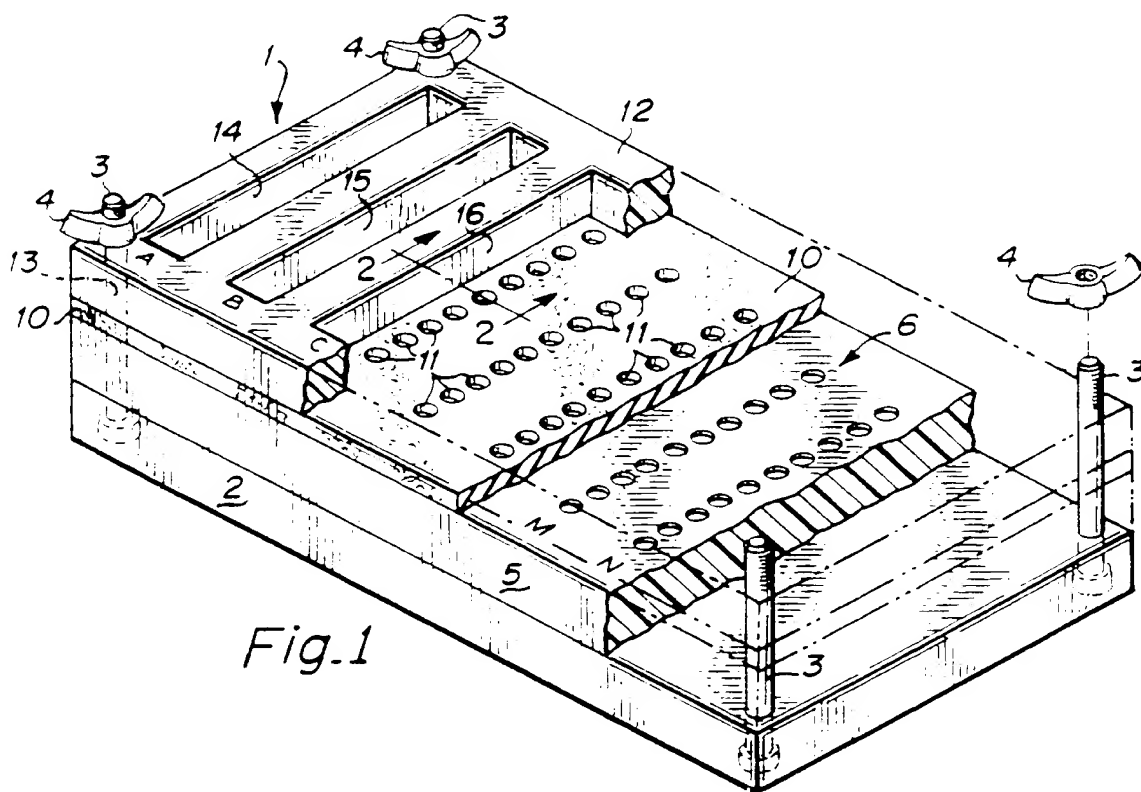
Fig.1
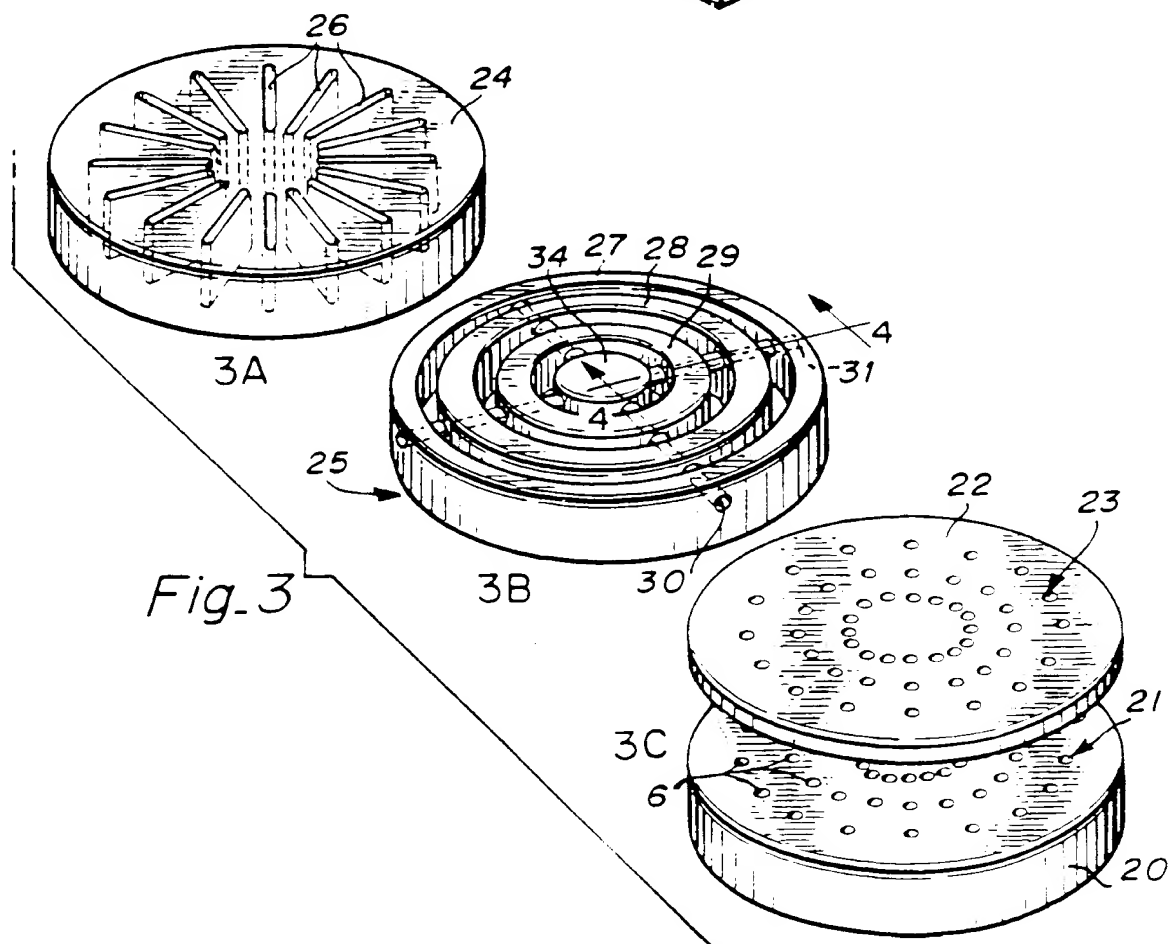
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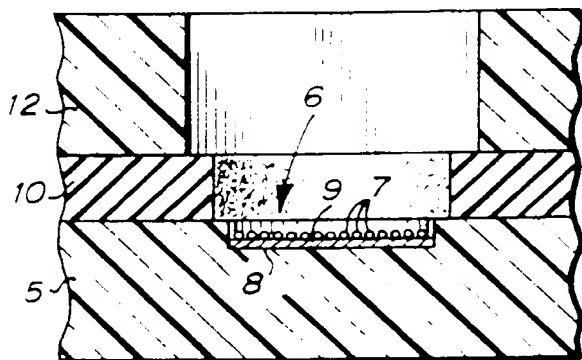
Fig_2a
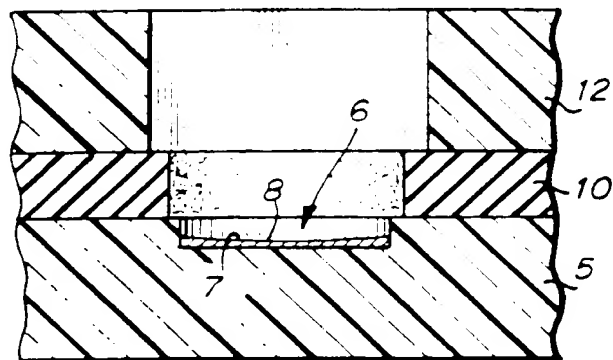
Fig_2b
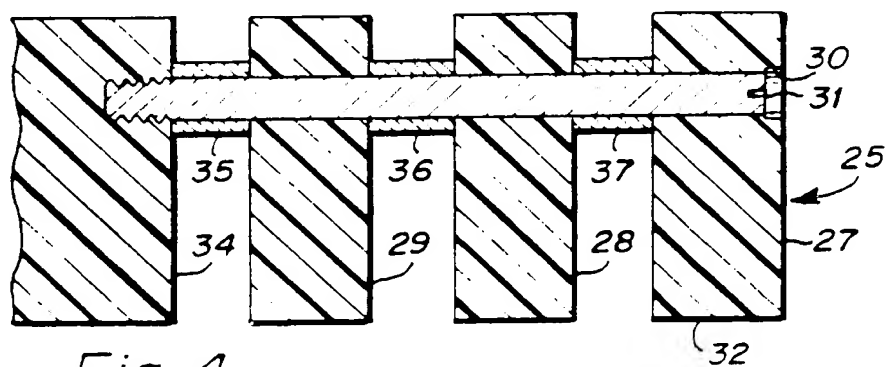
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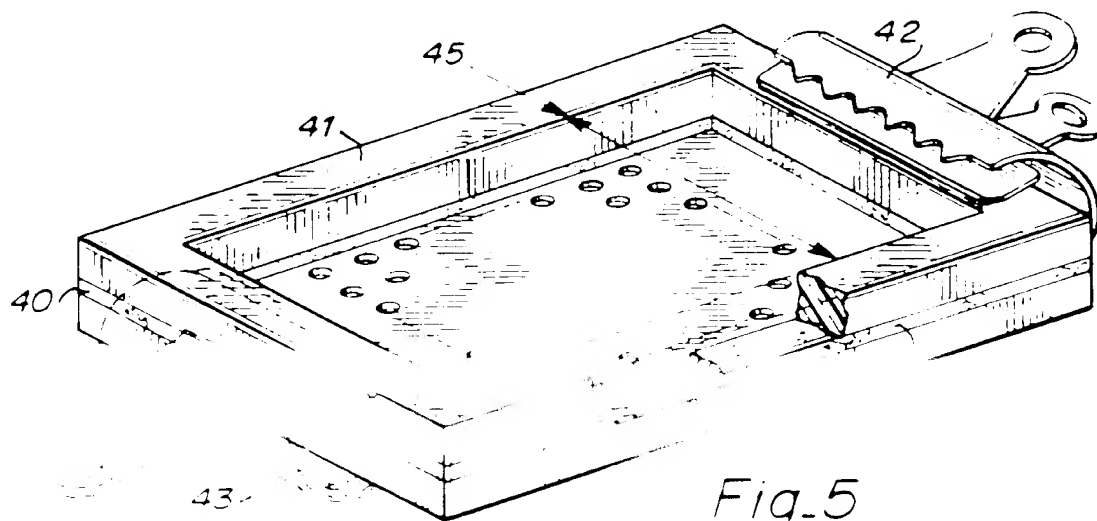
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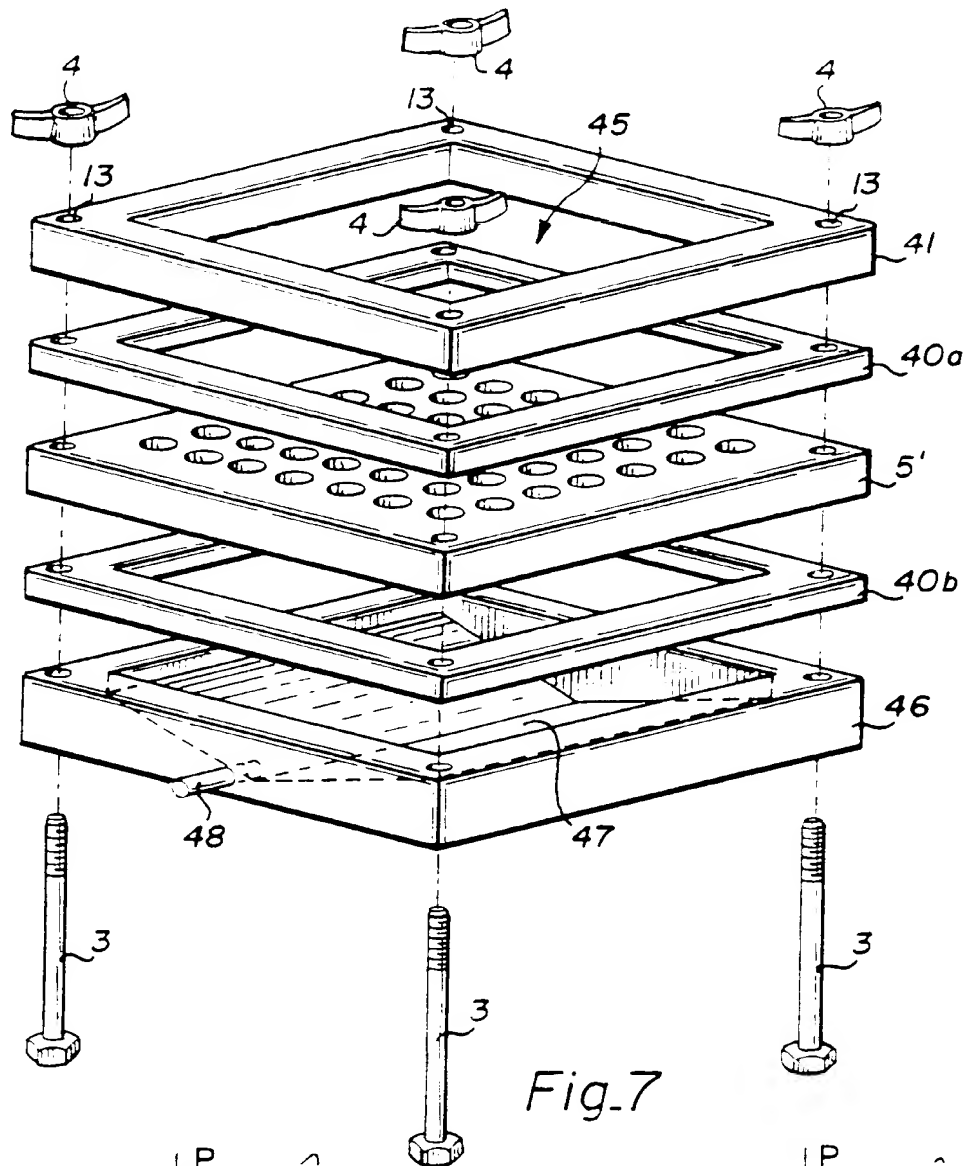
Fig. 7
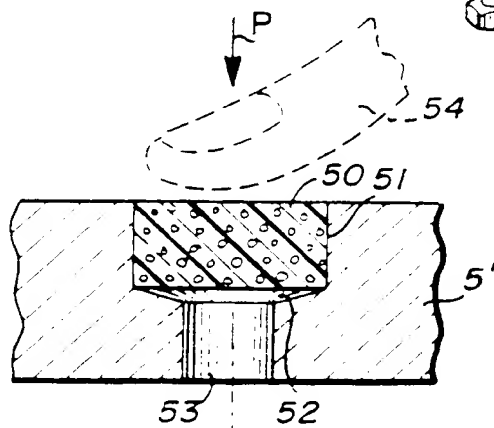
Fig. 8A

Fig. 8B

Fig.6

Fig.9

ASPARAGINE
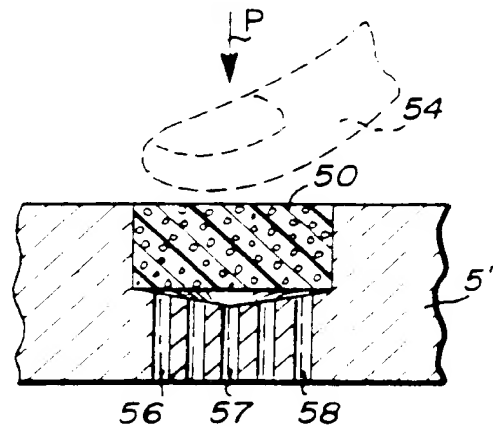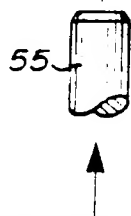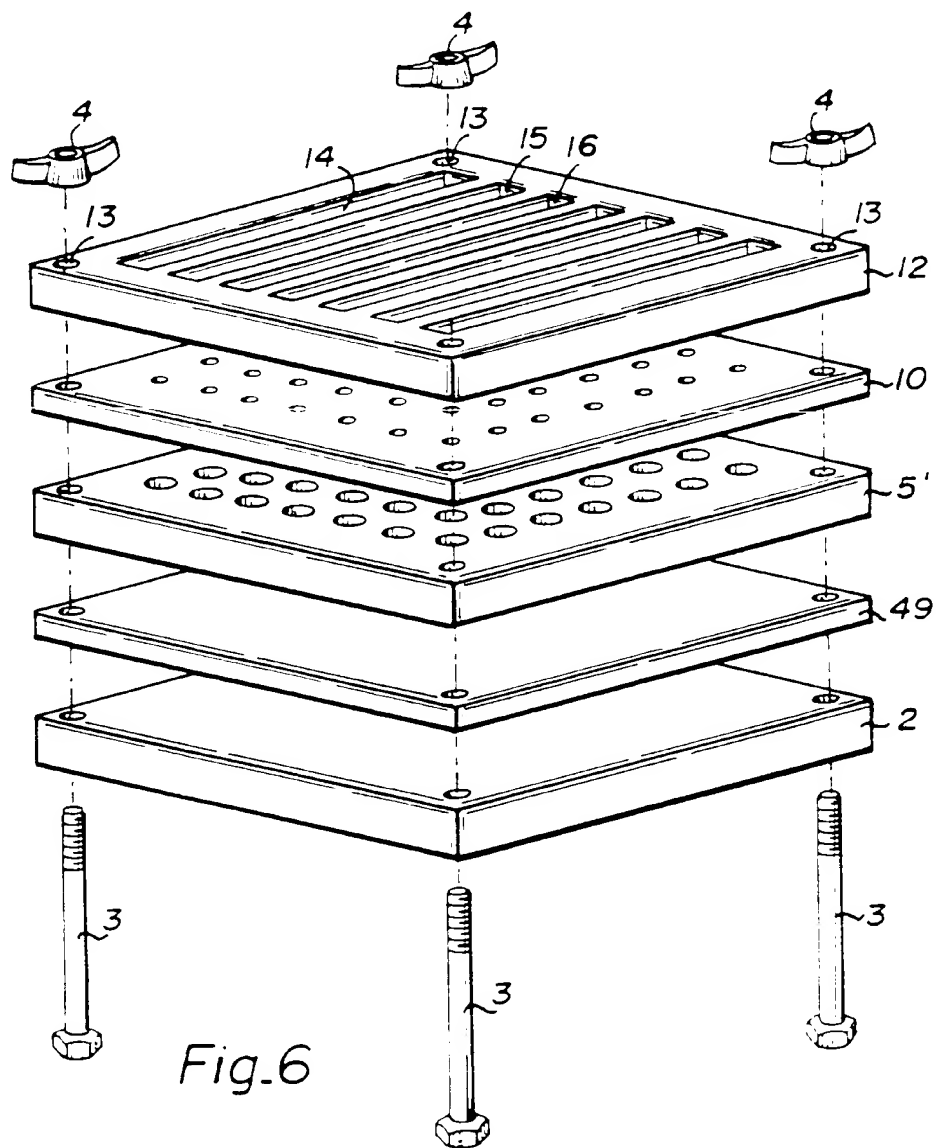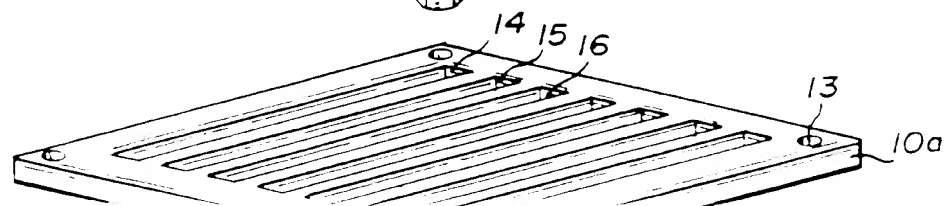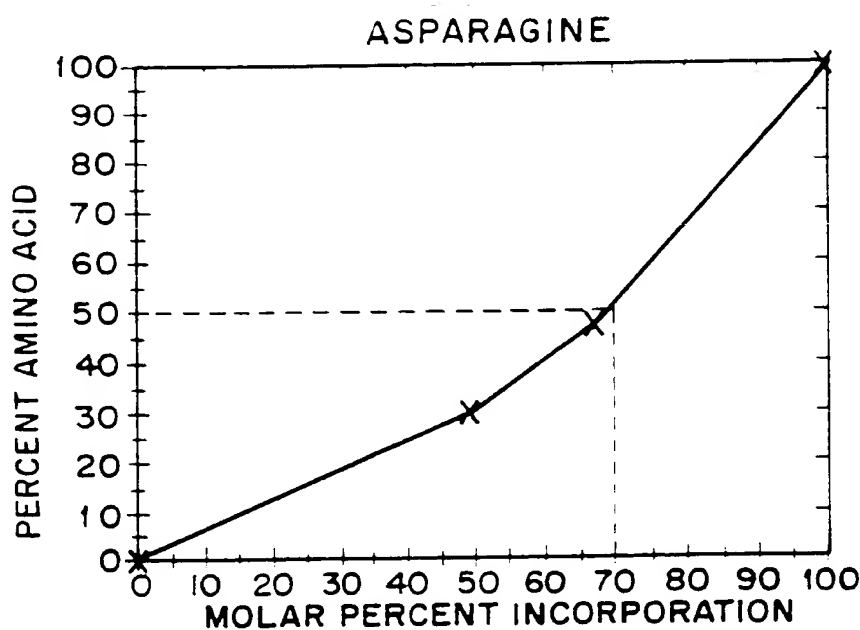
Fig. 10

ARRAY TEST 355 STREPTAVIDIN
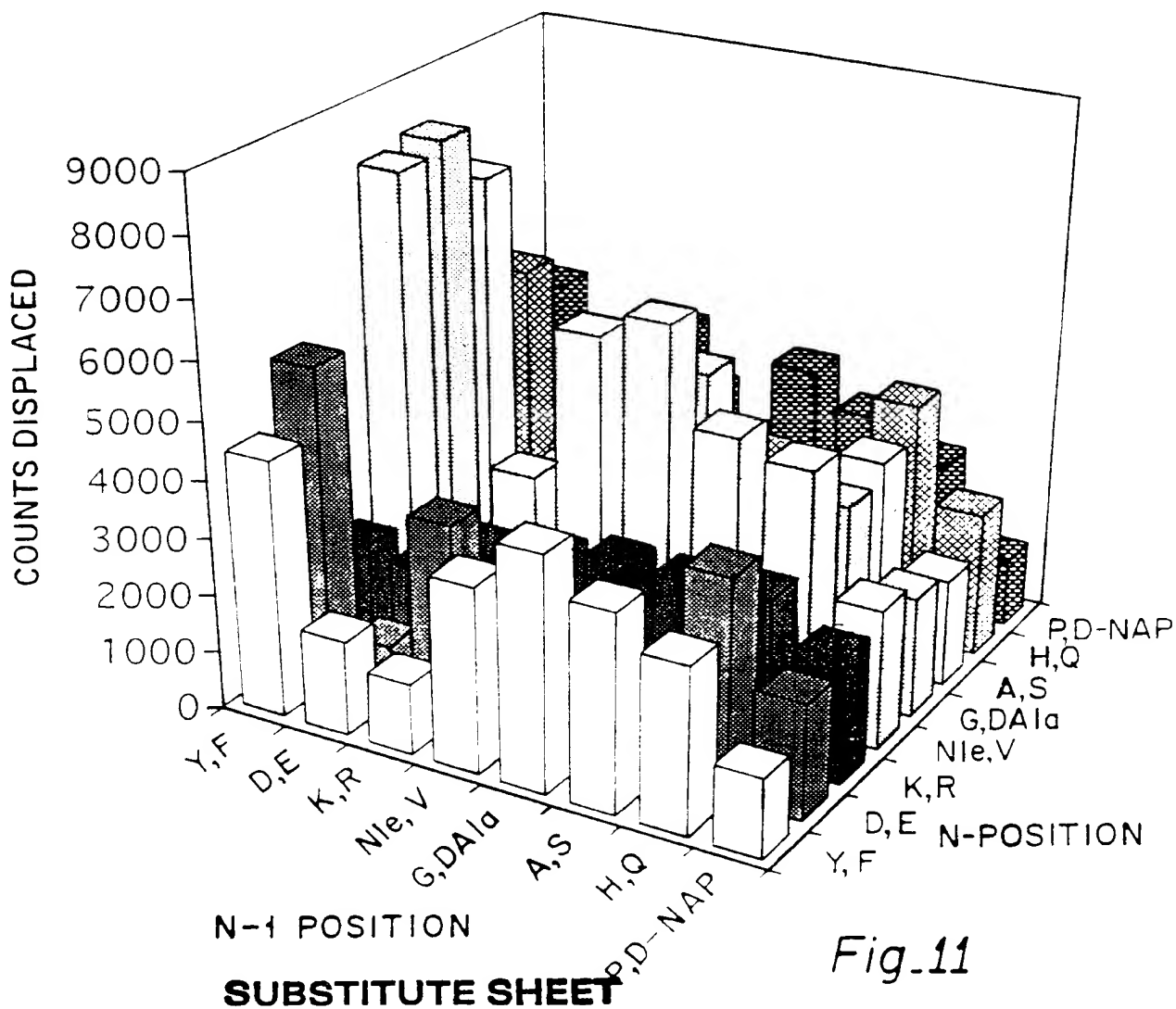
Fig. 11

SUBSTITUTE SHEET

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :Please See Extra Sheet.

US CL   :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,728,502 (Hamill) 01 March 1988, abstract, Fig. 4, col. 1, lines 49-66, col. 3, lines 13-26 and 58-62.	1-5, 8-11
X	US, A, 5,100,626 (Levin) 31 March 1992, abstract, Figs. 2-3, col. 3, lines 37-50, col. 4, lines 20-25 and 46-55, col. 5, line 18 - col. 6, line 11, col. 7, lines 25-40, col. 8, lines 35-42, col. 9, lines 8-20	16-18
Y		1, 4, 6, 8-9, 11, 14-15
X	WO, A, 90/02605 (Meldal et al) 22 March 1990, abstract, page 1, lines 3-9, page 3, lines 13-16, page 6, line 24 - page 7, line 27, page 12, line 29 - page 15, line 29, Figs. 3, 6-8 and 16.	1
Y		2-3, 9-13

[X] Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

*	Special categories of cited documents:	'T'	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'A'	document defining the general state of the art which is not considered to be part of particular relevance		
'E'	earlier document published on or after the international filing date	'X'	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'L'	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	'Y'	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
'O'	document referring to an oral disclosure, use, exhibition or other		

Date of the actual completion of the international search	Date of mailing of the international search report
01 DECEMBER 1993	1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ARDIN A SPIEGEL
Facsimile No. NOT APPLICABLE	Telephone No. 703) 308-0196

Form PCT ISA/210 (second sheet)(July 1992)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Genomics, volume 13, issued August 1992, E. Southern et al, "Analyzing and Comparing Nucleic Acid Sequences by Hybridization to Arrays of Oligonucleotides: Evaluation Using Experimental Models", pages 1008-1017, especially abstract, pages 1008-1011.	16-18 1-2, 4-5, 8-9, 11-15
X Y	US, A, 5,143,854 (Pirrung et al) 01 September 1992, abstract, Figs. 10A-10M, col. 3, line 9 - col. 4, line 27, col. 7, lines 49-62, col. 8, lines 1-7, col. 11, lines 31-38 and 51-68, col. 12, lines 43-45, col. 15, lines 9-22, col. 16, lines 18-21, 28-32 and 46-52, col. 17, line 66 - col. 18, line 3, col. 18, lines 40-68, col. 19, lines 34-37, col. 20, lnes 10-32, col. 23, lines 19-67, col. 24, lines 12-14.	1, 16-18 2-15
Y,P	US, A, 5,188,733 (Wang et al) 23 February 1993, Figs. 1 and 3-6, col. 2, line 38 - col. 3, line 3, col. 3, lines 25-33 and 43-61, col. 4, lines 17-19, 49-51 and 56-57, claims 1-8	1-4, 7, 9, 11, 14
Y	Journal of the American Chemical Society, vol. 111, no. 20, issued 27 September 1989, R. Berg et al, "Long-Chain Polystyrene-Grafted Polyethylene Film Matrix: A New Support for Solid-Phase Peptide Synthesis", pages 8024-8026, especially pages 8024-8025.	1-4
Y	Tetrahedron Letters, vol. 33, no. 21, issued 19 May 1992, M. Meldal et al, "PEGA: A Flow Stable Polyethylene Glycol Dimethyl Acrylamide Copolymer for Solid Phase Synthesis", pages 3077-3080, especially pages 3077-3078.	3
Y,P	Tetrahedron Letters, vol. 34, no. 10, issued 05 March 1993, S. Kates, "A Novel, Convenient, Three-Dimensionsal Orthogonal Strategy for Solid-Phase Synthesis of Cyclic Peptides", pages 1549-1552, especially page 1549.	9
A	US, A, 5,053,454 (Judd) 01 October 1991, see entire document.	1-18
A,P	US, A, 5,147,608 (Hudson et al) 15 September 1992, see entire document.	1-9
A,P	US, A, 5,175,209 (Beattie et al) 29 December 1992, see entire document.	1-18
A,P	US, A, 5,196,566 (Barany et al) 23 March 1993, see entire document.	1-18

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,258,454 (Berg et al) 02 November 1993, see entire document	1-8

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

B01D 25/12, 63/00; B01J 19/00; B01L 9/00, 11/00; C08F 283/00, 285/00; C07H 21/00; C07K 1/06, 5/00, 7/00, 15/00, 17,00; C08L 89/00; C12Q 1/00; C12P 19/04, 19/38, 21/06; G01N 21/00, 33/00, 33/543, 33/544, 33/545, 33/546, 33/552

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/321.84, 321.75, 323.1, 450, 638; 422/ 56, 57, 58, 101, 102, 103, 104, 130, 131, 134, 136, 191, 209; 427/2, 491; 435/7.1, 7.92, 7.94, 69.1, 188, 287, 293, 301, 312; 436/89, 94, 518, 527, 528, 529, 531, 532; 525/54.1, 54.11; 530/333, 334, 335, 336, 337

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

210/321.84, 321.75, 323.1, 450, 638; 422/ 56, 57, 58, 101, 102, 103, 104, 130, 131, 134, 136, 191, 209; 427/2, 491; 435/7.1, 7.92, 7.94, 69.1, 188, 287, 293, 301, 312, 961, 973; 436/89, 94, 518, 527, 528, 529, 531, 532, 807; 525/54.1, 54.11; 530/333, 334, 335, 336, 337 935, 79, 80, 81, 82, 87, 88

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG
search terms: solid phase synthesis or preparation, peptide, polypeptide, oligonucleotide, polysaccharide, amino or amine function? or derivati?, spacer, linker, handle, channel or slot block, gasket, seal, vacuum, polyolefin, polyethylene, dextran, polyethylene glycol, chitin, chitosan, polydimethylacrylamide, silica, polystyrene, crosslink?, kieselguhr

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I.   Claims 1-3 and 7, drawn to an apparatus comprising a solid phase polymeric support having a substrate matrix of individually defined amino-functionalized areas for chemically anchoring biopolymers thereto, wherein the substrate is preferrably a polymer film having low molar percentage of crosslinking covalently grafted to the support, classified in class 435, subclass 174..

II.   Claims 4-7, drawn to a channel block apparatus comprising a common well access to an inert support having a substrate matrix of individually defined areas for attaching biopolymers thereto, wherein the substrate preferrably comprises a hydrophilic polar multi-functionalized polymer film attached to a support preferrably comprising porous polyolefin discs, classified in class 422, subclass 131.

III.   Claim 8, drawn to an apparatus for synthesizing biopolymers comprising a support having individual holes containing a hydrophilic polar multi-functionalized polymer film attached porous polyolefin discs, sandwiched between an overlying border block and an underlying vacuum base, and a sealing means capable of providing fluid flow through the porous discs, classified in class 422, subclass 104.

IV.   Claims 9-13, drawn to methods for synthesizing polymers in an apparatus comprising a plurality of rotatable slot blocks and using random equimolar monomers and polymers comprising protected amino acids, protected nucleic acids and protected monosaccharides, classified in class 530, subclass 333.

V.   Claim 14, drawn to a method of synthesizing and individually screening an array of discrete polymer sequences on a substrate for reactivity against a preselected target, classified in class 436, subclass 518.

VI.   Claims 15-18, drawn to methods for screening a synthetic peptide combinatorial library in order to determine which polypeptides specifically bind to a known target receptor, classified in class 435, subclass 7.1.